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CLAIMS

What is claimed is:

1. An isolated nucleic acid molecule encoding a replication protein selected from the group consisting of:

(a) an isolated nucleic acid encoding the amino acid sequence as set forth in SEQ ID NO:2;

(b) an isolated nucleic acid that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; or

an isolated nucleic acid that is complementary to (a), or (b).

2. The isolated nucleic acid of Claim 1 as set forth in SEQ ID NO:1.

3. A polypeptide encoded by the isolated nucleic acid of Claim 1.

4. The polypeptide of Claim 3 as set forth in SEQ ID NO:2.

5. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 379 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:2, or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

6. A method of obtaining a nucleic acid molecule encoding an replication protein comprising:

(a) probing a genomic library with the nucleic acid molecule of any one of Claims 1 or 5;

(b) identifying a DNA clone that hybridizes with the nucleic acid molecule of any one of Claims 1 or 5; and

(c) sequencing the genomic fragment that comprises the clone identified in step (b),

wherein the sequenced genomic fragment encodes a replication protein.

7. A method of obtaining a nucleic acid molecule encoding a replication protein comprising:

(a) synthesizing an at least one oligonucleotide primer corresponding to a portion of the sequence as set forth in SEQ ID NO:2; and

(b) amplifying an insert present in a cloning vector using the oligonucleotide primer of step (a);

wherein the amplified insert encodes a portion of an amino acid sequence encoding a replication protein.

8. The product of the method of Claims 6 or 7.

9. An isolated nucleic acid molecule encoding a plasmid stability protein selected from the group consisting of:

(a) an isolated nucleic acid encoding the amino acid sequence as set forth in SEQ ID NO:4;

(b) an isolated nucleic acid that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; or

an isolated nucleic acid that is complementary to (a) or (b).

10. The isolated nucleic acid of Claim 9 as set forth in SEQ ID NO:3.

11. A polypeptide encoded by the isolated nucleic acid of Claim 9.

12. The polypeptide of Claim 11 as set forth in SEQ ID NO:4.

13. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 296 amino acids that has at least 70% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:4, or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

14. A method of obtaining a nucleic acid molecule encoding a plasmid stability protein comprising:

(a) probing a genomic library with the nucleic acid molecule of any one of Claims 9 or 13;

(b) identifying a DNA clone that hybridizes with the nucleic acid molecule of any one of Claims 9 or 13; and

(c) sequencing the genomic fragment that comprises the clone identified in step (b),

wherein the sequenced genomic fragment encodes a plasmid stability protein .

15. A method of obtaining a nucleic acid molecule encoding a plasmid stability protein comprising:

(a) synthesizing an at least one oligonucleotide primer corresponding to a portion of the sequence as set forth in SEQ ID NO:3;and

(b) amplifying an insert present in a cloning vector using the oligonucleotide primer of step (a);

wherein the amplified insert encodes a portion of an amino acid sequence encoding a plasmid stability protein.

16. The product of the method of Claims 14 or 15.

17. A plasmid comprising the nucleic acid of Claim 1.

18. A plasmid comprising the nucleic acid of Claim 1 and the nucleic acid of Claim 13.

19. A plasmid having the nucleotide sequence as set forth in SEQ ID NO:5.

20. A plasmid according to Claim 17 or 18 further comprising at least one nucleic acid encoding a selectable marker.

21. A plasmid according to Claim 19 wherein the selectable marker is selectable in both gram negative and gram positive bacteria.

22. A plasmid according to Claim 17 or 18 further comprising an origin of replication that is functional in a gram positive bacterium.

23. A plasmid according to Claim 22 wherein the gram positive bacterium is a member of the Actinomycetales bacterial family.

24. A plasmid according to Claim 23 wherein the gram positive bacterium is selected from the group consisting of, *Actinomyces, Actinoplanes, Arcanobacterium, Corynebacterium, Dietzia, Gordonia, Mycobacterium, Nocardia, Rhodococcus, Tsukamurella, Brevibacterium, Arthrobacter, Propionibacterium, Streptomyces, Micrococcus,* and *Micromonospora*.

25. The plasmid according to Claim 17 or 18 further comprising at least one promoter suitable for the expression of a gene in *Rhodococcus*.

26. A plasmid having the nucleotide sequence as set forth in SEQ ID NO:6.

27. A plasmid having the nucleotide sequence as set forth in SEQ ID NO:7.

28. A method for the expression of a nucleic acid in an *Actinomycetales* bacteria comprising:

a) providing a plasmid comprising:

(i) the nucleic acid of Claim 1 and the nucleic acid of Claim 13;

(ii) at least one nucleic acid encoding a selectable marker; and

(iii) at least one promoter operably linked to a nucleic acid fragment to be expressed;

b) transforming an *Actinomycetales* bacteria with the plasmid of (a); and

c) culturing the transformed *Actinomycetales* bacteria .of (b) for a length of time and under conditions whereby the nucleic acid fragment is expressed.

29. A method according to Claim 28 wherein the plasmid further comprises an origin of replication that is functional in gram positive bacterium.

30. A method according to Claim 29 wherein the selectable marker gene is selected from the group consisting of ampicillin resistance gene, tetracycline resistance gene, chloramphenicol resistance gene, kanamycin resistance gene, and thiostrepton resistance gene.

31. A method according to Claim 28 wherein the nucleic acid fragment to be expressed is selected from the group consisting of genes encoding; enzymes involved in the production of isoprenoid molecules, polyhydroxyalkanoic acid (PHA) synthases, carotenoid biosynthesis enzymes, nitrile hydratases, ethylene forming enzyme, pyruvate decarboxylase, alcohol dehydrogenase, terpene synthases, and cholesterol oxidase.

32. A method according to Claim 28 wherein the *Actinomycetales* bacteria is selected from the group consisting of *Actinomyces, Actinoplanes, Arcanobacterium, Corynebacterium, Dietzia, Gordonia, Mycobacterium, Nocardia, Rhodococcus, Tsukamurella, Brevibacterium, Arthrobacter, Propionibacterium, Streptomyces, Micrococcus,* and *Micromonospora.*

33. A method according to Claim 32 wherein the *Actinomycetales* bacteria is is selected from the group consisting of: *Rhodococcus equi, Rhodococcus erythropolis, Rhodococcus opacus, Rhodococcus rhodochrous, Rhodococcus globerulus, Rhodococcus koreensis,Rhodococcus fascians, and Rhodococcus ruber.*

34. A transformed bacteria comprising the plasmid of Claim 17 or 18.

35. A transformed bacteria according to Claim 34 wherein the bacteria is a member of the *Actinomycetales* bacterial family.

36. A transformed bacteria according to Claim 35 wherein the bacteria is selected from the group consisting of, *Actinomyces, Actinoplanes, Arcanobacterium, Corynebacterium, Dietzia, Gordonia, Mycobacterium, Nocardia, Rhodococcus, Tsukamurella, Brevibacterium, Arthrobacter, Propionibacterium, Streptomyces, Micrococcus,* and *Micromonospora*.

37. A transformed bacteria. according to Claim 36 selected from the group consisting of: *Rhodococcus equi, Rhodococcus erythropolis, Rhodococcus opacus, Rhodococcus rhodochrous, Rhodococcus globerulus, Rhodococcus koreensis, Rhodococcus fascians, and Rhodococcus ruber.*

38. A transformed bacteria of Claim 34 comprising a second plasmid belonging to a different incompatibility group.

39. A method for the expression of a nucleic acid in an *Actinomycetales* bacteria comprising:

a) providing a first plasmid comprising:
(i) the nucleic acid of Claim 1;
(ii) at least one nucleic acid encoding a selectable marker; and
(iii) at least one promoter operably linked to a nucleic acid fragment to be expressed;

b) providing at least one other plasmid in the different incompatibility group as the first plasmid, wherein the at least one other plasmid comprises:
(ii) at least one nucleic acid encoding a selectable marker; and
(iii) at least one promoter operably linked to a nucleic acid fragment to be expressed;

c) transforming an *Actinomycetales* bacteria with the plasmids of (a) and (b); and

d) culturing the transformed *Actinomycetales* bacteria of (c) for a length of time and under conditions whereby the nucleic acid fragment is expressed.

40. A method according to Claim 39 wherein the *Actinomycetales* bacteria is selected from the group consisting of *Actinomyces, Actinoplanes, Arcanobacterium, Corynebacterium, Dietzia, Gordonia, Mycobacterium, Nocardia, Rhodococcus, Tsukamurella, Brevibacterium,*

Arthrobacter, Propionibacterium, Streptomyces, Micrococcus, and *Micromonospora.*

41. A method according to Claim 39 wherein the at least one other plasmid is pDA7 having the ATCC designation ATCC 47072.